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EFFECTS OF DIFFERENT PRETREATMENTS AND SEED COLLECTION DATES OF ORIENTAL HORNBEAM (*Carpinus orientalis* Mill.) SEEDS ON GERMINATION

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ABSTRACT

Seeds of oriental hornbeam (*Carpinus orientalis* Mill.) were collected from the natural distribution area in Turkey. Seeds were collected from three different populations originating from three different altitude levels (0-400 m, 400-800 m, 800-1200 m), from the sea level to the highest point of the study sites (1200m).

Including control, 22 different pretreatments were applied on seeds to overcome seed dormancy originated from seed coat and embryo. Also, differences that were related to different seed collection dates and altitude were further examined. Seeds were also sown in a nursery, under open field conditions, to determine tree and population germination percentages.

Highest germination percentage (58.5%) was found in Tree no: 22 in population 1 while lowest germination percent (0.5%) was found in Tree no: 13 in population 3. Highest germination percentages of pretreatments were found in citric acid (5000) in population 1, GA3 (500 ppm) in population 2, and stratification 8 weeks + GA3 500 ppm in population 3. According to the study results germination differences within and between populations were determined.

KEYWORDS: *Carpinus orientalis* Mill., germination, seed pretreatment, seed collection date

1. INTRODUCTION

Carpinus orientalis Mill. is native to southeastern Europe to northern Iran and has wide natural distribution area. It usually can be found on dry lower altitudes than *Carpinus betulus* [1]. Natural distribution area of Oriental hornbeam in Turkey includes Thrace, Aegean, Marmara Region, Northern Anatolia and Eastern Anatolia [2, 3]. Oriental hornbeam has high drought tolerance and usually grows on dry, shallow and stony soils [4]. Thick bark, which reduces the mortality, is characteristic for this species [5]. It is resistant to drought stress which makes it suitable for degraded land rehabilitation [6]. It is also being used in landscape applications due to its pruning capabilities and contributions to biodiversity.

Hornbeam seeds have seed dormancy originated from seed coat, embryo and endosperm [7]. Also, because embryo of hornbeams is not always fully developed, seeds can stay in r seedbed for a long period of time and germination may occur in the following year [8].

Seed dormancy can be described as a situation where healthy and alive seeds cannot germinate under optimal conditions [9]. Seed dormancy is affected by seed physiology, seed characteristics, and environmental conditions. Dormancy can be originated from both embryonic activities and/or seed coat [10, 11]. There are different classifications of seed dormancy. Dormancy; A- Physiological dormancy, dormancy state of embryo, B- Morphological dormancy, premature embryo, C- Morphophysiological dormancy, premature embryo and embryo dormancy, D- Physical dormancy, seed coat impermeability, E- Combined dormancy (physical+physiological) [12].

Dirr and Heuser (1987) [13] recommended 3 months of cold stratification followed by 2 months of warm stratification in order to increase germination percentage of *C. orientalis*. In another study on *Carpinus orientalis,* the highest germination percentage (90.8%) was found by using 2000 ppm GA3 followed by 4 months of cold stratification [6].

Czapracki and Holubowicz (2010) [14] tested five different pretreatments on *Carpinus betulus* L. seeds. Peat + sand medium, 4 weeks 20-22°C warm stratification and 16 weeks in 3-5°C cold stratification. In another study on *Carpinus laxiflora, Carpinus tshonoskii,* and *Carpinus cordata* species cold stratification was successful in dormancy breakage as germination percentage of *C. laxiflora* and *C. tshonoskii* was above 80%. [15].

Guo (2000) [16] examining *Carpinus cordata* var. *chinensis* and *Carpinus fargesiana* seeds germination, reported that these two species have dormancy state for one

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or more years since they showed no germination in first year but only in the second and third growth season.

Seed collection date for hornbeam species is around late September and early October [7, 17]. Hartmann et al. (1997) [18] reported that hornbeam seeds should be collected early autumn (September-October) and sown in autumn or spring while Dirr and Heuser (1987) [13] reported that there were no germination in seeds that were sown in autumn without any pretreatment. Hornbeam seeds can be collected when they turn brown and before they are fully hardened in September to easily overcome dormancy caused by seed coat.

In the present study, we test the hypothesis that different pretreatments, altitude and seed collection date may affect seed dormancy. Therefore, we tested 22 different pretreatments to overcome seed dormancy in relation to populations found in different altitudes. Changes in germination percentage, related to seed collection date, were also examined.

2. MATERIALS AND METHOD

2.1 Information on the Studied Populations and Trees

Seed material of this study was collected from Trabzon-Maçka watershed where is a natural distribution area of oriental hornbeam (*Carpinus orientalis* Mill.). Optimum seed maturation period for oriental hornbeam is late September and early October, thus, study material was collected at this period. Seeds were collected from three different altitude levels (0-400 m, 400-800 m, 800-1200 m), from sea level to the highest point of the watershed, and collected from a total of 43 trees. Seeds were labeled according to population and individual they belong. Seeds were then prepared for pretreatments; these preparations are wiping out foreign materials from seeds and separating seeds from their pericarps. Table 1 contains information about the selected study sites, the populations, the general aspect, the soil type and the geological substrate [19] of the studied populations and Figure 1 shows the geographical location.

2.2 Germination tests

2.2.1 Pretreatments

Several pretreatments were applied on oriental hornbeam seeds to overcome seed dormancy which is originated from seed coat and embryo as shown in Table 2. Control treatment was applied on full seeds without pericarp, which passed the alcohol floating method.

TABLE 2 - The different pretreatments for *Carpinus orientalis* seeds

Pre-treatment
Control
Seed tip cut
GA_3 (100,250, 500ppm)
Citric Acid (5000, 10000, 15000ppm)
Hot water (10,30,60s)
H_2SO_4 (30,60,180min)
Stratification 4,8,12 weeks
Stratification 4,8,12 weeks+GA3 500 ppm

Number of seeds that were used were: 12600 for pretreatments (3 populations x 20 pretreatments x 3 replications x 70 seeds), 9450 for trees (3 populations x 15 trees x 3 replications x 70 seeds), 3150 for populations (15 trees x 3 replication x 70 seeds) and 900 for collection date (3 collection date x 3 replication x 100 seeds).

After the pretreatment of the seeds, they were sown using randomized sampling design in open field seedbeds in October. Table 3 shows the meteorological information of the open field seedbeds.

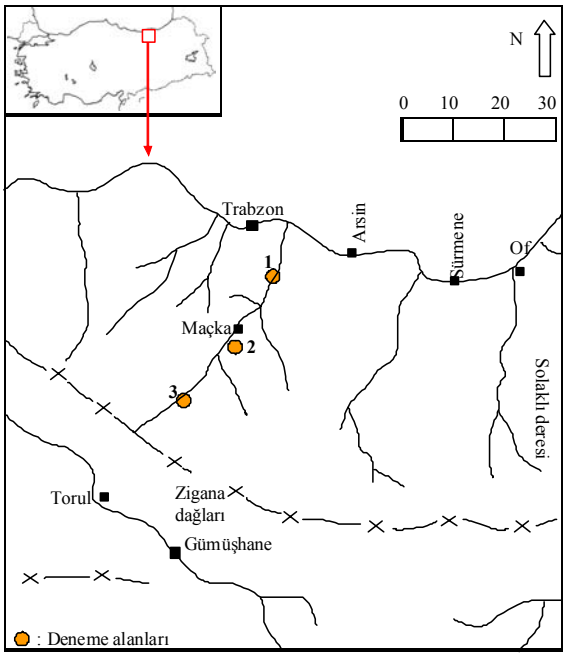

FIGURE 1 - Geographical location of study populations

TABLE 1 - Study site information

Pop. No	Population Name	Number of Trees (N)	*Eastern Longitude	*Northern Latitude	Altitude (m) asl.	General aspect	Soil type	Geological substrate
1	Maçka 1	13	558705-558991	4524593-4525691	178-335	East, Northeast	Brown earths	Basalt, Andesite
2	Maçka 2	15	552358-557613	4509344-4519865	450-776	East, South, Southwest	Brown earths	Basalt, Andesite
3	Maçka 3	15	550029-558991	4507115-4509575	800-1130	South, west, Northwest	Brown earths	Granite

TABLE 3 - Meteorological information of the open field seedbeds

Months (2012)	Average Temperature	Average Moisture	Total precipitation	Months (2012)	Average Temperature	Average Moisture	Total precipitation
February	5˚C	70 %	19.3 mm	June	23˚C	74 %	91.3 mm
March	5˚C	73 %	45.2 mm	July	25˚C	72 %	20.4 mm
April	13˚C	71 %	43.0 mm	August	25˚C	70 %	23.0 mm
May	18˚C	79 %	47.2 mm	September	22˚C	72 %	32.6 mm

In order to determine the effect of seed collection date on germination seeds were collected in three different collection dates, August 15th 2011, September 15th 2011, and October 15th 2011. Seeds were collected from one population (Maçka 2).

To find out the effect of altitude on seed germination, seeds were collected from there different population found in three different altitudes ranging from 0-1200 m.

2.3 Statistical Analyses

SPSS program was used to determine the significant differences between measured characteristics. Mean percentage of seed germination based on pretreatments and populations were determined. Two way analysis of variance was conducted to determine significant differences between germination percentages of pretreatments, populations, and population x pretreatments. Duncan test was conducted to determine grouping of pretreatments and populations.

3. RESULTS

3.1 Results of Pretreatments

Pretreatments were applied on seeds of 43 trees from three different altitudes of Maçka watershed. Seed fullness was determined with alcohol floating method. Full seeds were used in germination tests. Table 4 contains germination percentage of all trees in populations sorted, from high to low.

According to Table 4 highest germination percent (58.5%) was found in Tree no: 22 in population 1 while lowest germination percent (0.5%) was found in Tree no: 13 in population 3.

Results of the different pretreatments that were applied on seeds to overcome seed dormancy are shown in Table 5.

Highest germination percentage for first population was found in "citric acid (5000) while "GA_3 (500 ppm)" pretreatment showed highest germination in second population. Stratification 8 week+GA3 500 ppm showed the highest germination percent in the third population. Soaking in hot water (90˚C) pretreatment showed no germination in any stage. Lowest germination percentages were found in control for the first and third population while H_2SO_4 (180 min) showed lowest germination percentage in the second population.

As for the different pretreatments, Duncan test created 9 different groups. 4 week stratification + GA3, 12 week stratification + GA3 and 12 week stratification were in same group. Another group was Cutting tip of the seed, control and H_2SO_4 (180 min). 8 week stratification and H_2SO_4 (60 min) were in another group while GA3 (100 ppm) and Citric Acid (5000 ppm) were in another group. Another group was GA3 (500 ppm) and 8 week stratification + GA3. Another group was also created by 4 week stratification and Citric Acid (10000 ppm). Other pretreatments created their own groups each by themselves.

Duncan test results showed that all populations were in same group (Table 5). Although there is no statistical difference between populations, it was observed that germination percent decreased as altitude increased.

Table 6 contains the highest germination percentages of populations and the difference between control results.

According to Table 4, differences between control and highest germination percentages change between 34.7% - 85.8%. This shows that some pretreatments greatly increase germination percentages.

TABLE 4 - Germination percentage of every tree in the three populations

Pop-Tree	Germination percent (%)	Pop-Tree	Germination percent (%)
1-22	58.5	1-28	17.0
1-27	44.5	3-39	16.0
1-26	38.5	3-45	14.5
3-36	36.0	2-6	13.5
1-20	35.0	3-44	13.5
2-33	35.0	3-42	12.5
1-21	34.5	2-8	12.0
3-37	34.5	3-11	12.0
2-34	33.5	2-3	11.0
1-17	32.0	2-35	10.0
2-31	32.0	3-40	9.0
1-14	30.0	3-38	8.5
1-19	27.5	2-2	8.0
2-30	27.5	2-4	8.0
1-18	27.0	3-12	7.5
1-15	26.0	1-24	5.5
1-25	25.5	3-10	3.0
2-32	25.0	3-9	1.0
3-43	24.5	3-41	1.0
2-5	21.0	2-7	0.5
2-29	19.0	3-13	0.5
2-1	18.0		
Mean			% 19.03

Population 1: 0-400m, Population 2: 400-800m, Population 3: 800-1200m

TABLE 5 - Germination percentage of the various pretreatments within and among populations

Pre-treatment	**P1	**P2	**P3	Average GP (%)
		Germ. percent		
Control	9.2	15.7	4.5	9.8 abc*
Seed tip cut	10.0	9.3	10.0	9.8 abc
GA_3(100ppm)	18.7	18.7	22.7	20.0 def
GA_3(250ppm)	24.7	8.0	18.0	16.9 bcdef
GA_3(500ppm)	20.7	24.0	18.7	21.1 ef
H_2SO_4 (30min)	10.5	9.1	7.0	8.8 ab
H_2SO_4 (60min)	16.7	12.7	9.2	12.8 abcd
H_2SO_4(180min)	21.7	3.0	6.6	10.4abc
Citric Acid (5000ppm)	36.7	11.7	13.4	20.6 def
Citric Acid (10000ppm)	34.7	22.3	12.7	23.2 f
Citric Acid (15000ppm)	28.0	18.7	10.0	18.9 cdef
Stratification 4 week	23.3	22.7	22.7	22.9 f
Stratification 4 week+GA3 500 ppm	3.3	4.0	4.0	3.8 a
Stratification 8 week	9.3	6.7	18.0	11.7 abcd
Stratification 8 week+GA3 500 ppm	18.7	14.7	32.0	21.8 ef
Stratification 12 week	1.3	14.7	5.3	7.1 a
Stratification 12 week+GA3 500 ppm	2.0	7.3	4.7	4.7a
P (Significant level)	17.0a	13.1a	12.9a	<0.01

(P:Population) P1: 0-400 m, P2: 400-800 m, P3: 800-1200 m *in p<0.01, ** in p>0.05

TABLE 6 - Some data related to germination percentages of populations

Populations	Highest Germination Percent (%)	Control Germination Percent (%)	Difference Between Control and Highest Germination Percentages (%)
1st Population	36.7	9.2	74.8
2nd Population	24.0	15.7	34.7
3rd Population	32.0	4.5	85.8

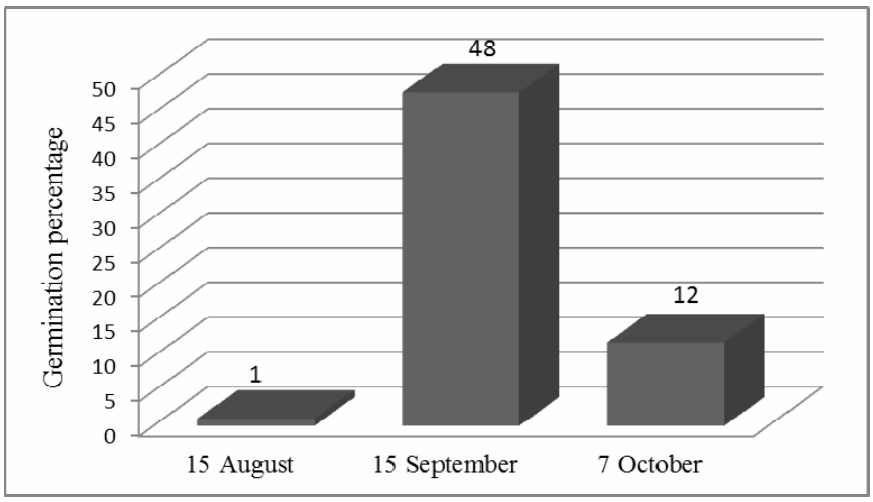

FIGURE 2 - Germination percentages for different seed collection dates

3.2 Germination Results Depending on Seed Collection Dates

As the effects of seed collection dates on seed germination were examined it was found that germination started in spring. Figure 2 contains the germination percentages.

Highest germination percentage was recorded in the seeds collected in the middle of September (48%) while lowest germination was observed in the seeds collected in August. According to these results the seed collection date plays a significant role on germination percentages.

4. DISCUSSION

Among pretreatments citric acid (10000 ppm), stratification for 4 weeks, and stratification for 8 weeks + GA3 500 ppm pretreatments showed the best germination percentages. Similar studies showed that cold stratification is one of the most effective methods in increasing germination percentage. Takos et al. (2001) [20] also reported that cold stratification for 3 months is enough for overcoming seed dormancy and increased germination percentage.

Treatments such as "cutting the tip of seed" and "H_2SO_4" showed low germination percentages. This may be caused by seed damage by these pretreatments. There are similar study results that show that some mechanical and chemical pretreatments can damage hornbeam seeds. Merou et al. (2012) reported [21] that *Carpinus orientalis* seeds that had chemical (H_2SO_4) and mechanical scarification showed lower germination percentages. This indicates that mechanical scarification can be deadly for seeds while chemical applications can harm the embryo. Similarly in a different study on *Carpinus orientalis* seeds, mechanical scarification by sand paper and immersion in 500 ppm (GA3) for 24 h in the dark at 25°C showed germination percentage of 2,5% and 4%, respectively. There was no germination in soaking in concentrated (98%) H_2SO_4 for 30 min and 1 hour and moist-cold stratification (4°C) for 12 weeks treatment. Reason of this thought to be the harmful effects of keeping seeds in acid which could damage seed embryos [22].

In a study testing the effect of gibberellic acid and stratification on *Carpinus caroliniana* seeds it was found that stratification increased germination percentage while GA3 was effective only to stratified seeds [23]. Our study results showed similarities. 500 ppm GA3 treatment on seeds after 8 weeks of stratification showed almost twice the germination percentage of seeds that were stratified for 8 weeks . However, 4 and 12 weeks of stratification + GA3 500 ppm treatments showed lower germination percent than only 4 and 12 weeks of stratification applied seeds. Similar to our results Takos et al. (2001) [20] reported that chemical or mechanical pretreatments before cold stratification reduce germination.

Bhatt and Ram (2007) [24] seeds that were collected from different altitudes were investigated. showed that seeds of *Carpinus viminea* Wall that were collected from higher altitudes had higher germination percentages than seeds collected from lower altitudes. However, in our study seeds from medium e (400-800 m) and higher (800-1200 m) altitude showed similar germination percentages while highest germination percent was found in seeds from the lowest altitude level (0-400 m). The results are probably due to the nursery place as the altitude of the nursery was 50 m which is similar to the altitude of the population that these seeds were collected. Thus, similar environmental conditions might have contributed to germination.

According to previous studies, seed collection date affect seed germination. In our study the highest germination was observed in the seeds that were collected in September.

5. CONCLUSIONS

Germination took place under open field conditions after pretreatments were applied. Germination percentage was evaluated in accordance with populations, trees within populations, and pretreatments. It was determined that populations and trees in populations also affected germination along with pretreatments. Variations based on germination characteristics of *Carpinus orientalis* Mill seeds that were collected from natural populations of high altitudes were determined.

Among seed collection dates, September showed the highest germination percentage. Thus, it can be said that higher germination can be observed when seeds are collected in the period where their pericarps turning from green to yellow.

Highest germination percentages of pretreatments were found in citric acid (5000) in population 1, GA3 (500 ppm) in population 2, and stratification 2 months + GA3 500ppm in population 3. Germination of Oriental hornbeam can be increased with stratification and GA3 treatments. Seed germination showed a trend to reduce as altitude increased.

Results showed that variations within populations are more than variations between populations, thus, determining the variations within population would be better. In order to achieve this, source of this variation within population should be determined by studying individuals in that population with analyses such as DNA, isoenyzme, etc.

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REFERENCES

[1] Bobrov, E.G. (1970). Carpinus L., In Flora of the U.S.S.R., (ed. N. Landau), vol. 5, pp. 202-207, Keter Press, Jerusalem, Israel.

[2] Yaltırık, F. (1982). Flora of Turkey and Aest Eagen Island, 7, 684, Editor P.H. Davis, Universty Press, Edinburgh.

[3] Demirci, A. (2006). Silvikültürün Temel İlkeleri, KTÜ Orman Fakültesi, Ders Notları Serisi No: 83, Trabzon.

[4] Browicz, G. (1982). Chronology of trees and shrubs in southwest Asia and Adjacent Regions, Vol. 1, Polish Scientific Publishers, Warszawa-Poland, 172 pp.

[5] Banj Shafiei, A., Akbarinia, M., Jalali, G. and Hosseini, M. (2010). Forest fire effects in beech dominated mountain forest of Iran. Forest Ecology and Management, 259, 2191-2196.

[6] Pipinis, E., Milios, E., Kiamos, N., Mavrokordopoulou, O. and Simiris, P. (2012). Effect of stratification and pretreatment with gibberellic acid on seed germination of two *Carpinus* Species. Seed Science and Technology, 40, 21-31.

[7] Pijut, P.M., (2008). *Carpinus*. IN: Woody Plant Seed Manual, Agriculture Handbook 727, Bonner, F.T. and Karrfalt, R.P. (eds.), USDA Forest Service, Washington, DC. pp. 328-332.

[8] Yahyaoğlu, Z. and Ölmez, Z., (2005). Tohum Teknolojisi ve Fidanlık Tekniği, Kafkas Üniversitesi, Artvin Orman Fakültesi, KAÜ Yayın No:1, Artvin Yayın No:1, 142s, Artvin.

[9] Bewley J.D., (1997). Seed Germination and Dormancy, Plant Cell, 9, 1055-1066.

[10] Koornneef, M., Bentsink, L. and Hilhorst, H., (2002). Seed Dormancy and Germination, Deeepartment of Plant Sciences, Wageningen University, 5, 1, 33-36.

[11] Black, M., Bewley, J.D. and Halmer, P., (2008). The Encyclopedia of Seeds: Science, Technology and Uses, CAB international, Wallingford.

[12] Baskin, C.C. and Baskin, J.M., (1998). Seeds: Ecology, Biogeography and Evolation of Dormancy and Germination, Academic Press, San Diego, California.

[13] Dirr, M. and Heuser., CH. W. JR., (1987). The Reference Manual of Woody Plant Propagation: From Seed to Tissue Culture. Varsity Press, Inc., Athens, Georgia. 239.

[14] Czapracki, M. and Holubowicz, R., (2010). Some factors ınfluencing the germination of the common hornbeam *Carpinus betulus* L. seeds, Bulletion UASVM Horticulture, 67, 1.

[15] Suzuki, W., (2000). Germination Traits and Adaptive Regeneration Strategies of the Three *Carpinus* Species, J. For. Res. 5, 181-185.

[16] Guo, K., (2000). Germination, Seedling, Survival and Growth of the Two *Carpinus* Species From a Chinese Beech Forest, Acta Phytoecologia Sinica, 24 ,4, 385-390.

[17] Saatçioğlu, F., (1971). Orman Ağacı Tohumları, İ.Ü. Yayın No:1649, Orman Fakültesi Yayın No:173, İstanbul.

[18] Hartmann, H., Kester T., Davies D.E. and Geneve R.L. (1997). Plant Propagation. Principles and practices. 5th Edition, Prentice-Hall International.

[19] Anonimuous, (2006). General Directorate of Mineral Research and Exploration, General Geological Structure of Trabzon.

[20] Takos, I., Konstantinidou, E and Merou, T., (2001). Effects of stratification and scarification on germination of Christ's thorn (*Paliurus spina-christi* Mill.) and oriental hornbeam (*Carpinus orientalis* Mill.) seeds. Proceedings International Conference Forest Research: a challenge for an integrated European approach, Thessaloniki, Greece, 27 August-1 September 2001, Volume I 2001 pp. 437-443

[21] Merou, T., Takos, I., Varsamis, G. and Xofis, P., (2012). Effect of stratification and scarification treatments on the germination of oriental hornbeam (*Carpinus orientalis*) Seeds, Seed Science and Technology, 40, 265-270.

[22] Tsitsoni, T., Tsakaldimi, M., and Tsouri, C., 2013. Seed treatments to break dormancy and stimulate germination in *Cercis siliquastrum* L. and *Carpinus orientalis* Mill., African Journal of Agricultural Research, Vol. 8(35), pp. 4501-4505.

[23] Breztloff, L.V. and Pellett, N.E., (1979). Effect of Stratification and Gibberellic Acid on the Germination of *Carpinus caroliniana* Walt., Hortscience, 14, 5, 621-622.

[24] Bhatt, j. and Ram, J., (2007). Seed and Seedling Characteristics of the Himalayan Hornbeam (*Carpinus viminea* Wall.), India, Ecol Res, 22, 156–159.

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